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Research Article

METHOD DEVELOPMENT AND VALIDATION OF DOXORUBICIN HCL IN API AND ITS FORMULATION BY SPECTROPHOTOMETRY

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ABSTRACT

The article describes simple, economical, sensitive and accurate Spectrophotometric Method for the estimation of Doxorubicine hydrochloride, anti-cancer chemotherapy drug, in bulk and pharmaceutical dosage form. The developed Method was based on the formation complex of Doxorubicine hydrochloride with Bromo phenol blue chromogenic reagent. The colored chromogen shows maximum absorption at 420nm. The absorbance-concentration plot is linear over the range 5-60µg/ml. The correlation co-efficient was found to be 0.998. The different experimental parameters affecting the development and stability were studied carefully and optimized. A result of analysis for the method was validated statistically and recovery studies were also performed.

Keywords: Doxorubicine hydrochloride, Bromo phenol blue.

1. INTRODUCTION

Doxorubicin hydrochloride trade nameAdriamycin RDF, or Rubex, chemically(7S,9S)-7-[(2R,4S,5S,6S)-4-amino-5-hydroxy-6-methyloxan-2-yl]oxy-6,9,11-trihydroxy-9-(2-hydroxyacetyl)-4-methoxy-8,10-dihydro-7H-tetracene-5,12-dione(). The structural formula is $C_{27}H_{29}NO_{11}$·HCl and molecular weight is 543.52 g/mol.Drug is soluble in water.Doxorubicin is known to interact with DNA by intercalation and inhibition of macromolecular biosynthesis. This inhibits the progression of the enzyme topoisomerase II, which unwinds DNA for transcription. Doxorubicin stabilizes the topoisomerase II complex after it has broken the DNA chain for replication, preventing the DNA double helix from being resealed and thereby stopping the process of replication. It is an anthracyclineantibiotic commonly used in the treatment of a wide range of cancers, including hematological malignancies, many types of carcinoma, breast cancer and soft tissue sarcomas.

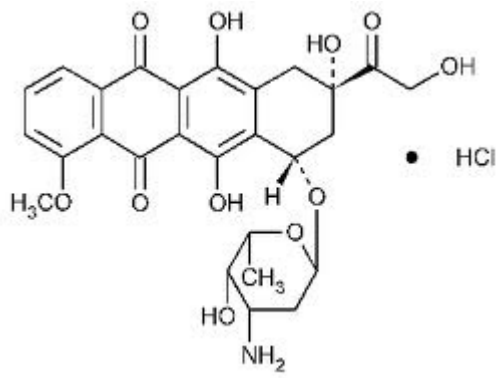


Fig. 1: structure of doxorubicin hydrochloride

No method has not been reported in bulk and in pharmaceutical formulationby usingUltra Violet –Visible double beam Spectrophotometer.Thus the present study was undertaken to develop and validate a simple, sensitive, accurate, precise, and reproducible UV spectrophotometric method for estimation of doxorubicin hydrochloride.

2. MATERIALS AND METHODS

2.1 MATERIALS USED

Apparatus

Elico double beam Ultra Violet –Visible double beam Spectrophotometer SL-244 with 1cm matched quartz cells was used for all spectral measurements.

Chemicals and Reagents

All the chemicals & reagents used were of analytical grade. All the solutions were freshly prepared.

- Bromophenol blue 0.05%
- Chloroform AR grade
- Methanol AR grade
- Phosphate buffer pH 3.6

➢ **Bromophenol blue (BPB) 0.05% w/v** - 0.1gm of dye sample while gently heating in 1.5ml of 0.1M NaOH and dissolve 20ml of methanol (95%), and make up to 100ml with distilled water.

➢ **Phosphate Buffer pH 3.6**: Dissolve 0.900gm of anhydrous di sodium hydrogen phosphate and 1.298gm of citric- acid monohydrate in sufficient water to produce 1000ml.

2.2 Preparation of standard stock solution

A standard stock solution containing 1 mg/ml was prepared by dissolving 100mg of doxorubicin hydrochloride in 100ml of water.

2.3 Preparation of working standard solution

From stock solution 10 ml was further diluted to 100 ml with distilled water to get the solution having concentration 100 µg/ml.

2.4 Determination of absorption maximum

From the above working standard solution, 5 ml was transferred into a 10 ml volumetric flask and the volume was made up to the mark with distilled water to prepare a concentration of 500 µg/ml. Then the sample was scanned in UV-VIS Spectrophotometer in the range 200-400nm using reagent blank and the wavelength corresponding to maximum absorbance (λ_{max}) was found to be 420nm.

2.5 Preparation of calibration curve

Aliquots of standard drug solution of Doxorubicin containing 0.1-1.0 ml (100 mcg/ml) are taken and transferred into series of graduated test tubes. To each test tube 2 ml of 0.05% w/v Bromophenol blue and 3 ml of Phosphate buffer pH 3.6 were added. Reaction mixture was shaken gently for 2 min. Then 5ml of chloroform was added to each of them. The solutions were shaken for 2 to 3 minutes and kept aside for the formation of colored complex. The absorbance of the yellow colored chromogen was measured at 420 nm against reagent blank and a calibration curve was constructed as depicted in Figure 2 & 3. The absorbance of the sample solution was measured, and the amount of the drug was determined by referring to the calibration curve or computed from the regression equation.

2.6 Assay of marketed formulation

Doxorubicin hydrochloride is available in injection vials. Each ml of vial contains 2mg of doxorubicin hydrochloride was successfully analyzed by the proposed methods. drug equivalent to 10mg of doxorubicin hydrochloridewas dissolved in 100ml of water and filteredand the final volume was made to 100ml with water to obtain 100ug/ml concentration. The solution was suitable diluted and analyzed as given under the assay procedure for bulk samples. The results are represented in table 2. None of the excipients usually employed in the formulation of drug interfered in the analysis of doxorubicin hydrochloride, by the proposed method.

METHOD VALIDATION

The developed method was validated as per ICH guidelines.

Linearity

The linearity was studied at 420nm in the concentration range of 5-60 µg/ml.

Accuracy

The % mean recovery obtained for doxorubicin was 99.8% .The %RSD is less than 2, results were given in Table 2.

Recovery studies

To ensure the accuracy and reproducibility of the results obtained, adding known amounts of pure drug to the previously analyzed formulated samples and these samples were reanalyzed by the proposed method and also performed recovery experiments. The percentage recoveries thus obtained were given in Table 2.

Specificity and selectivity

The spectra obtained from injection solutions were identical when compared with the standard solution containing same concentration of doxorubicin. This showed that there was no any interference from excipients. Therefore, it could be said that developed method is highly selective.

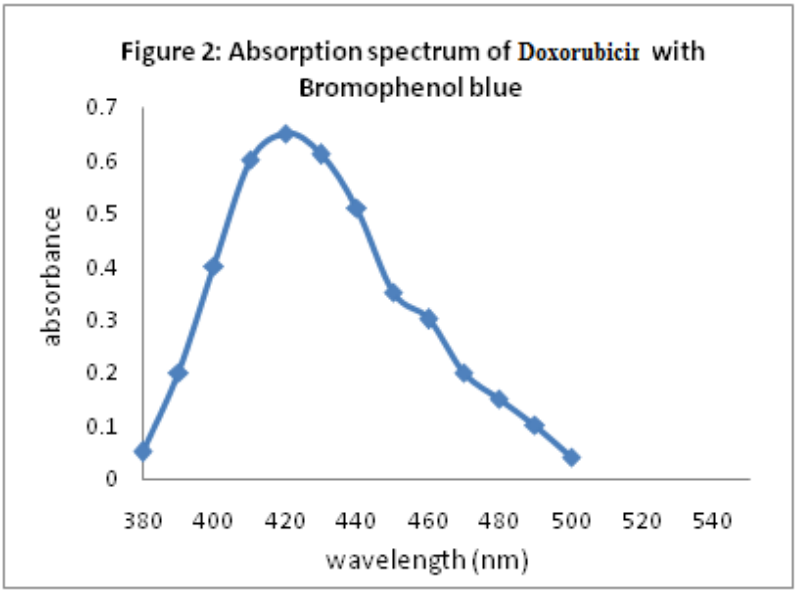
Detection limit and quantification limit

LOD for Doxorubicin was 0.06µg/ml respectively, while LOQ was 0.2µg/ml.

3. RESULTS AND DISCUSSION

In the present work a method has been developed for the estimation of doxorubicin hydrochloride frominjection formulation. The developed method was based on formation of colored complex with Bromo phenol blue.The condition required for formation of colored complex was optimized. Statistical analysis was carried out and the results were satisfactory. Relative standard deviation values were low that indicates the reproducibility of the proposed methods. Recovery studies were close to 100% that indicates the accuracy and precision of the proposed methods. The optical characteristics such as absorption maxima, beer's law limit, molor-absorptivity and sand ell's sensitivity are presented in table 1. The regression analysis using the method of least square was made for slope (m), intercept (b) and correlation obtained from different concentrations and the results are summarized in table 1.

In conclusion, the proposed method are simple, economical, sensitive, precise reliable and reproducible for the routine estimation of doxorubicin hydrochloride in bulk as well as in injection formulation.



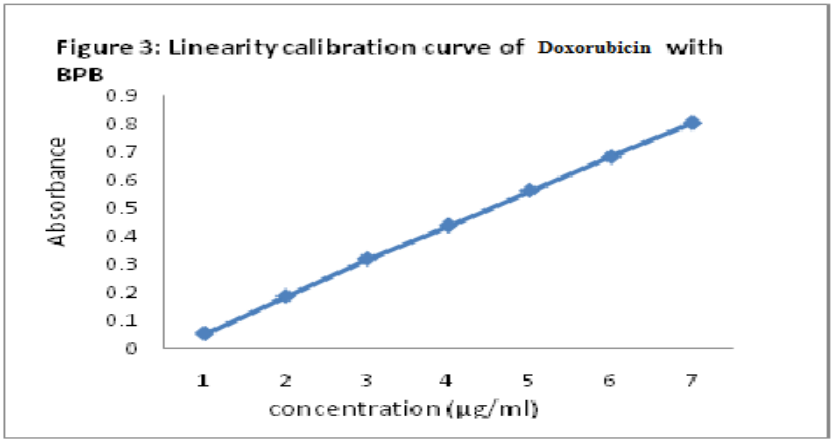


Table 1: regression and validation parameters of doxorubicin HCl regression parameters

S. No.	Parameter	Result
1	Slope (m)	0.004
2	Intercept (c)	0.0012
3	% RSD	0.6042
4	Standard Regression Equation	Y=0.004x+0.0012
5	Correlation Coefficient (R)	0.998
6	Standard error of estimate	0.0294
7	Confidence intervals (upper limit=1)	0.971-0.991

validation parameters

1	Absorption maxima(nm)	420
2	Beers law limit (µg/ml)	5-60
3	Molar absorptivity (micrograms/cm2/0.001absorbance unit)	9.7×10^3
4	Sand ell's sensitivity (micrograms/cm2/0.001absorbance unit)	0.6280
5	Limit of Detection (mcg/ml)	0.066
6	Limit of Quantification (mcg/ml)	0.2
7	Optimum photometric range (µg/ml)	5-50
8	Color stability (hours)	1.5
9	Accuracy(% Recovery ±SD)	99.8±0.22

Table 2: Assay of doxorubicin HCL in injection formulation

Injection formulation	labeled amount (mg)	amount obtained(mg)* by proposed method	%recovery by the proposed method **
01	10	9.7 ±0.31	97 ±0.14
02	10	9.98±0.12	99.8±0.22
03	10	9.47 ±0.26	94.7±0.34

*average of three determinations **after spiking the sample

CONCLUSION

A spectrophotometric method for quantifying doxorubicin HCl in formulation samples has been developed and validated. The assay is selective, precise, accurate and linear over the concentration range studied. In summary, the proposed method can be used for the drug analysis in routine quality control.

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